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BOX PATENT APPLICATION
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Washington, D.C. 20231

Sir:
Transmitted herewith for filing is the patent application of:

Inventor(s): Edward SHANBROM

For: METHOD FOR QUANTIFYING ANTIOXIDANT LEVELS IN FOODS AND MEDICAL SPECIMENS

Enclosed are:
18 Pages of Specification
___ Sheet(s) of drawing (__formal __ informal)
X Declaration and Power of Attorney __ Will follow.
X Form PTO-1595 and an assignment of the invention to SHANBROM TECHNOLOGIES LLC, of 603 West Ojai Avenue, Suite 5, Ojai, California 93023-3732 . __ Will follow
__ A certified copy of _____ from which priority is claimed in the subject case pursuant to Rule 55b and 35 U.S.C. 119. __ Will follow.
__ An associate Power of Attorney
X A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27.
__ Information Disclosure Statement, Form PTO 1449, and __ cited reference(s).
__ Preliminary Amendment
X General authorization/request to Petition for Extensions of Time
X Return Postcard

FOR.	NO. FILED	NO EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE		OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE				$380 00	OR		$ 760 00
TOTAL CLAIMS	5-20=	-0-	X 9	$	OR	X 18	$
INDEPENDENT CLAIMS	3 -3=	-0-	X 39	$	OR	X 78	$
___ MULTIPLE DEPENDENT CLAIMS PRESENTED			X+ 130	$	OR	+ 260	$
			TOTAL =	**$380.00**		**TOTAL:**	**$**

__ Please charge my Deposit Account No. 07-1853 the amount of $_____. A duplicate copy of this sheet is enclosed.
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__ The issue fee set in 37 CFR 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 CFR 1.311(b).
__ Any filing fees under CFR 1.16 for presentation or extra claims.

Respectfully submitted,

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VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 C.F.R. 1.9(f) & 1.27(c)) - SMALL BUSINESS CONCERN	PATENT DOCKET NO: 38786.00069

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Edward SHANBROM

Serial No.: Currently unknown

Filed: Concurrently herewith

Title: METHOD FOR QUANTIFYING ANTIOXIDANT LEVELS IN FOODS AND MEDICAL SPECIMENS

I hereby declare that I am

____ the owner of the small business concern identified below:

__X__ an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Small Business Concern: Shanbrom Technologies LLC

Address of Small Business Concern: 603 West Ojai Avenue, Suite B, Ojai, CA 93023-3732

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18 and reproduced in 37 CFR 1.9(d) for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year; and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to, and remain with, the small business concern identified above with regard to the invention described in:

__X__ the specification filed herewith with title as listed above.

____ the application identified above.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights in the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c), if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR (1.9(d), or a non-profit organization under 37 CFR(1.9(e).

Each person, concern or organization having any rights in the invention is listed below:

__X__ No such person, concern or organization exists.

____ Each such person, concern or organization is listed below.

Full Name:

Address:

____Individual ____Small Business Concern ____Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon or any patent to which this verified statement is directed.

Dated: May 17, 1999

By: _____

Name: William J. Shanbrom

Title: President

Address: 603 West Ojai Avenue, Suite B, Ojai, CA 93023-3732

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PATENT APPLICATION

38786.00069

Method for Quantifying Antioxidant Levels In Foods and Medical Specimens

Edward Shanbrom

Method for Quantifying Antioxidant Levels In Foods and Medical Specimens

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present application concerns biology and biological chemistry and more particularly measurement of antioxidant properties of foods and biological specimens.

2. Description of Related Art

Recently there has been a revolution in human nutrition and our understanding of the relationship between human health and diet. Most of us take for granted that foods contain vitamins and that these compounds are responsible for many of the beneficial properties of food. However, this belief is a recent one. The first vitamin to be scientifically identified and described was vitamin A (retinol) which was not described until 1913. The most recently identified of the "core" vitamins is vitamin B_{12} which was not described until 1948. Thus, all of the major vitamins were not described until the first half of this century although the properties of certain vitamins were known considerably earlier. The knowledge that fresh fruits (particularly citrus fruits) contain some factor that prevents scurvy goes back at least several hundred years. Apocryphally, the practice of the British Navy of providing limes to their sailors on long ship voyages to prevent scurvy is responsible for the common nickname of "limeys" given to British sailor.

It seems likely that all of the "vitamins" (meaning important food factors beyond fats, carbohydrates and proteins) have not yet been discovered. This is probably at least partially responsible for the current interest in "herbal medicine" and functional foods wherein ingestion of various natural products are supposed to have particular health benefits. The studies on heart disease over the last thirty years also make it clear that not only is it important to add vitamins and other factors to the diet, it is also important to avoid certain food substances that were formerly supposed to be benign. In particular the ingestion of saturated fats, generally of animal origin, has been shown to result in artery damage and an overall lessening of cardiac fitness.

It was with some surprise that the anti-fat crusaders discovered that certain Southern European diets that are exceptionally high in saturated fats do not produce the same degree of heart disease as do fatty diets in the United States. This finding led to a search for a "protective factor" to neutralize the baneful effects of dietary fats. Several candidates rapidly came to the forefront. The Mediterranean diets are not only high in saturated fats from meats and cheeses, they are also high in mono-unsaturated fats, particularly from olive oil. There are some studies that suggest that mono or poly-unsaturated fats can at least partially neutralize the harmful effects of saturated fats. At the same time the European diet also includes a significant amount of alcohol usually in the form of red wine. There is some evidence that moderate alcohol consumption has an ameliorating influence on the circulatory system.

More importantly, perhaps, tannins or polyphenols found in red wine are powerful antioxidants. There is growing evidence that dietary antioxidants prevent a number of maladies including heart disease. Antioxidant vitamins such as vitamin C and vitamin E are strongly implicated in the prevention of heart disease and a number of other diseases. Certainly, the dietary suggestion of at least five servings per day of fruits and vegetable

provides abundant antioxidants in the form of antioxidant vitamins as well as antioxidant polyphenolic compounds.

Studies of human nutrition have shown that a shortage of dietary antioxidants results in "oxidative stress" in which uncontrolled free radical production results in oxidative damage to proteins, lipids and nucleic acids. Antioxidants provide a biochemical environment that does not favor production of free radicals. Further, any free radicals that are formed are rapidly neutralized by antioxidants. Therefore, it is not surprising that a number of techniques have been developed to measure the presence of antioxidants. It is desirable to measure the antioxidant capacity of various foods as a way of estimating potential benefit from various foods or food additives. It is also beneficial to measure antioxidant levels of blood, urine, and other medical samples to asses the antioxidant status of a given patient and to determine the effect of ingesting various antioxidants on that antioxidant status.

A number of different analytic tests are used to determine antioxidant and/or "free radical trapping" content of foods. To the extent that many important antioxidants are polyphenolic compounds test, such as bromine reduction tests, for polyphenols are employed. A series of other tests are used to determine free radical trapping. The total (peroxyl) radical-trapping antioxidant potential (TRAP) assay employs 2'-azobis-(2'amidinopropane)-dihydrochloride to initiate formation of peroxyl radicals in a test substance. An oxygen electrode is then used to measure the rate that a given lipid sample resists peroxidation (e.g., by measuring the rate of oxygen uptake) in the presence of various test antioxidants. This method is generally most effective at measuring lipid soluble antioxidants and does not give a straightforward antioxidant value.

The trolox-equivalent antioxidant capacity (TEAC) is another method that measures the ability of antioxidants to prevent or quench free radicals. 2,2'azinobis-(3-

ethylbenzothiazoline-6-sulfonic acid) (ABTS) is reacted with hydrogen peroxide in the presence of a peroxidase enzyme to form radical cations whose presence can be detected optically by their effect on 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox). The method measures the inhibition of free radicals by antioxidants in the sample. This method requires several fairly complex reagents.

The oxygen radical absorbance capacity (ORAC) assay uses AAPH to generate peroxyl radicals. The radicals are optically measured as the fluorescence quenching and/or destruction of the algal pigment β-phycoerythrin. The assay can be automated allowing the free-radical quenching power of samples to be analyzed. Generally, instruments capable of fairly sophisticated optical measurements (e.g., fluorescence life time or total fluoresecence measurements) are required.

The total oxyradical scavaging species (TOSC) assay quantifies the reactive oxygen species scavaging potential of antioxidants. In this test ABAP is thermally decomposed to generate peroxy radicals which in turn generate ethylene gas by oxidatively decomposing α-keto-γ-methiobutyric acid. Antioxidants that scavenge the reactive oxygen prevent or diminish the formation of ethylene which is measured by a gas chromatograph. Again fairly complex and sophisticated instrumentation is required. Other complex analytic equipment such as electron spin resonance (ESR) spectrometers can also be employed to measure scavenging of free radicals by antioxidants.

Antioxidants can be measured in plasma using the fairly simple FRAP oxidation-reduction assay which optically measures the reduction of ferric-tripyridyltriazine to a ferrous form. This test is somewhat simpler than the toxic bromine test mentioned above.

SUMMARY OF THE INVENTION

The present invention uses a simple redox reaction to measure the antioxidant level of foods and medical specimens such as urine. A stable, non-toxic complex of iodine is added to the material to be measured. Antioxidants in the sample to be measured reduce elemental iodine to iodide ion which is readily measured with a highly sensitive iodide electrode. The instrumentation involved is extremely simple since the iodide electrode is extremely rugged and can be used with pH meters and similar analytic instruments. Because portable meters are readily available, the test can be readily used in field work as well as laboratory analysis. Although the test is primarily intended to measure water soluble antioxidants, it can also measure fat soluble antioxidants albeit more slowly. This is a result of the hydrophobic properties of elemental iodine. If the iodine reagent is emulsified with an organic phase, the iodine will transfer into the organic phase and undergo reduction by any antioxidants present. If the emulsion is "broken" (e.g., through addition of a wetting or antifoaming agent) or mixed with an aqueous phase, the resulting iodide ions will transfer into the aqueous phase and may be measured therein. Another approach to measuring fat soluble antioxidants is to dissolve them in a water miscible organic liquid such as alcohol (methanol, ethanol, propanol or butanol), benzyl alcohol or glyme ethers, etc. The PVP-I reagent can be mixed with these solvents so that the iodine dissolves. After the reaction, the solvent is mixed with water to facilitate measurement of the iodide ions.

DETAILED DESCRIPTION

OF THE PREFERRED EMBODIMENTS

The following description is provided to enable any person skilled in the art to make and use the invention and sets forth the best modes contemplated by the inventor of carrying out his invention. Various modifications, however, will remain readily apparent to those skilled in the art, since the general principles of the present invention have been defined herein specifically to provide an iodine-based redox measurement of antioxidants.

As already mentioned, the present test is based on the reduction of elemental iodine by antioxidant compounds. In this process each iodine atom receives an electron and becomes an iodide ion. According to standard oxidation-reduction reactions the iodine will be reduced by any compound that has a standard oxidation reduction potential that is more negative than the +.54 volt half cell potential of an iodine/iodide cell. This oxidation reduction range includes many important antioxidants including vitamin C and tannins.

In theory the reactive elemental iodine could be provided in a variety of different forms. However, iodine is relatively insoluble in aqueous solutions. The tri-iodide (e.g., iodine + iodide) is readily soluble but it is relatively corrosive and somewhat toxic (although not nearly as toxic as complex organic chemicals used in some antioxidant tests). Therefore, the present test can be carried out with tri-iodide solutions (Lugol's solution), but this is not the preferred method because of the higher iodide background and because of the potential toxicity of the reagent. Elemental iodine can be rendered water soluble and essentially non-toxic by any of a number of iodine coordinating materials which are also known as iodophors. The most popular iodophor is probably polyvinylpyrrolidone (povidone). An iodine-polyvinylpyrrolidone complex (povidone

iodine or PVP-I) is commercially available. The method of the present invention employs a 10% aqueous solution of PVP-I. Because the PVP-I is about 10% by weight elemental iodine in the dry state, the 10% reagent solution provides 1% elemental iodine by weight. Of course, a range of PVP-I concentrations are useable and grades containing different percentage weights of iodine can also be used.

The method of the present invention is as simple as adding an aliquot of the PVP-I reagent to an antioxidant solution and measuring the conversion of iodine to iodide with an iodide electrode. Of course, the method depends on an excess of iodine so that all available antioxidant will be oxidized. This is readily achieved by measuring serial dilutions of the unknown substance. When the dilutions produce a linear result (e.g., a one to one dilution produces one half as much iodide ion), one is assured that the proper excess of iodine is being maintained. A simple way of assuring that adequate iodine is present to guarantee linearity is to observe the color or the reaction solution. The initial PVP-I solution has a fairly intense red/brown color. As the reaction proceeds, colorless iodide is formed so that the overall color fades. If the solution becomes entirely clear, then insufficient iodine was present to result in a correct measurement. The sample should be diluted and the experiment repeated. The visible lightening of the color of the solution is an indication that the sample contains a potent antioxidant. The method can be readily calibrated by measuring known amounts of a reducing agent such a vitamin C or sodium bisulfite.

A significant advantage of the method is the ability to carry out the test almost anywhere with simple reagents and compact equipment. The preferred method for measuring iodide concentration is a portable ion selective electrode (ISE) and meter such as those manufactured by Orion Instruments, Inc. After calibration of the electrode by establishing a calibration curve through the use of a known concentration of antioxidant

(reducing agent), the experimental substance can be measured. As already explained, the method works by measuring the formation of iodide ions as the sample antioxidant reduces iodine in the PVP-I reagent. However, the sample may contain iodide to begin with, and the PVP-I reagent does contain iodide in the absence of any antioxidant. These additional sources of iodide must be corrected for. The experimental results are expressed as Iodine Reducing Units (IRU/volume) wherein the IRU value represents final iodide concentration at completion of the reaction (in parts per million). The IRU value is corrected by subtracting any iodide reading in the sample prior to the addition of the PVP-I reagent and by subtracting the iodide reading of the PVP-I reagent without addition of any antioxidant sample. Since iodine reduction is directly related to antioxidant capacity, IRUs can legitimately be called "AntiOxidant units" or AO units. A material with twice the antioxidant capacity of another material will show an IRU reading that is twice as great.

Example 1; Effect Of Dietary Antioxidants On Urine

This experiment was undertaken to determine the effect, if any, on the ingestion of dietary antioxidants on the antioxidant status of urine. In other words, if antioxidant materials are eaten, is there a significant excretion of antioxidants? For this experiment a highly concentrated polyphenolic antioxidant prepared from cranberries was used (trade named SHANSTAR™). The subject ingested a three gram sample of the cranberry antioxidant. A reference urine sample was taken a time zero and at 30 min intervals for the first two hours and then hourly until six hours had elapsed.

For each measurement a 25 ml aliquot of urine was placed in a 50 ml tube and any background iodide present was determined. Then 2.5 ml of 10% PVP-I was added and the solution mixed thoroughly. The rise in iodide concentration was measured over a period of 30 min. Earlier experiments had determined that the reaction reaches completion within 30 min under these conditions. Finally, the effective iodide concentration of the PVP-I

reagent was determined by adding 2.5 ml of PVP-I reagent to 25 ml of deionized water. The corrected (measured value minus initial iodide reading and minus reagent iodide reading) IRU values are shown in Table 1, below.

Table 1.

Time	Corrected IRU/ml
0 min	796
30 min	1416
60 min	1666
90 min	1766
2 hr	1316
3 hr	786
4 hr	666
5 hr	566
6 hr*	4116

* The 6 hr measurement was taken following ingestion of vegetable soup for lunch.

These results demonstrate an unusually rapid effect of increase in urinary antioxidant level following ingestion of antioxidants. It appears that excretion starts within 30 min of ingestion. Considering the rapidity with which the antioxidant impacts the urine it seems likely that absorption is directly into the blood stream through the buccal, esophageal or even stomach mucosa. It would also appear that peak secretion occurs within one and one half hours of ingestion and that values have returned to their base level within about 3 hr. Interestingly, the overall antioxidant level appears to decrease between meals thereby suggesting a possible value to between meal ingestion of antioxidant snacks. Clearly, vegetable soup is an excellent source of urinary antioxidant. It was noted that peak antioxidant levels coincided with a darkening (yellowing) of the urine color.

Example 2; Additional Measurements of Dietary Antioxidants On Urine

The measurements of urine were repeated after determining the background antioxidant level of the subject's urine. Urine was measured as explained under Experiment 1. Over a four day period the IRU was determined at the same time each day. The reading varied between 200 and 220 IRU. Measurement showed that the urine always had a pH of 6.0. The subject then ingested 500 mg of concentrated cranberry antioxidant. The results are shown in Table 2.

Table 2.

Time	pH	Corrected IRU/ml
ingestion	6.0	200
1 hr 45 min	6.0	920
3 hr 15 min	5.5	1220
4 hr 15 min	5.5	1300
7 hr 15 min	5.5	1320
17 hr 15 min	5.5	1330
19 hr 15 min	5.5	1300
24 hr 45 min	6.0	1120
28 hr 15 min	6.0	900

This experiment covered a much greater period of time than the initial experiment. In addition, a much smaller dose of the antioxidant was taken as a capsule which would mitigate against buccal or esophageal absorption. It is likely that this form of administration slowed absorption of the material. Further, the composition was formulated with cellulose which might prolong the release of the material. The subject did not fast over the experimental period so that some of the readings may be influenced by other antioxidants from ingested food.

Example 3; Antioxidant Level of Fruit Juices

For this experiment a number of different fruit juices were measured. Of course, commercial juices contain various concentrations of actual juice. Since these numbers were not normalized against "100%" juice, they are not truly comparable with each other. The juices had an initial iodide value of essentially zero. Therefore, the only correction needed was that for the iodide reading of the PVP-I reagent. As an indication of reaction rates, Table 3 shows corrected meter readings at 1, 5, 15 and 30 min. After 30 min there was little further change in iodide concentration.

Table 3.

Juice	1 min	5 min	15 min	30 min
white grape	97	375	501	535
apple	466	756	826	1056
blackberry[1]	481	846	1236	1496
blueberry[1]	339	703	856	936
raspberry	1216	1786	2426	2376
cranberry	263	475	746	836

Sample was diluted 1:10 before measurement.

These results show that of these commercially available juices both blackberry and blueberry had by far the highest level of antioxidant (reducing agent). Note that the reaction slopes of the different juices varies considerably. Raspberry shows a relatively shallow slope due to a high IRU reading at one minute. Experiment has shown that simple soluble antioxidants such as vitamin C react very rapidly with the PVP-I reagent while complex tannins react more slowly. This would suggest that the raspberry juice has a very high level of vitamin C or a similar factor.

Example 4; Antioxidant Level of Other Plant-Based Food Products

Twenty five ml aliquots of commercial carrot juice and red wine ("burgundy") were measured as shown above. In addition, measurements were made of dried figs. One gram

of finely chopped dried fig (chopped with a food processor) was suspended in 25 ml of water and incubated at room temperature for 60 min. Then the resulting solution was measured using the method of the current invention. The results are shown in Table 4

Table 4.

Food	Corrected IRU/ml
red wine	110
carrot juice	70
dried fig	1430

As might be expected, the red wine contains a reasonably high level of antioxidants. The dried figs contains a surprisingly large amount of antioxidant. This comparison is somewhat hard to make because ideally the weight of solutes in each tested material would be known. It is possible that on a weight of solute basis the red wine contain the greatest amount of antioxidant.

Example 5; Antioxidant Level of Eggs

Recently some egg producers have supplemented the feed of their laying hens with vitamin E to produce eggs that supposedly have enhanced antioxidant products. For this experiment "enhanced" eggs and regular eggs were measured using the method of the present invention. Four different egg samples were measured: a) liquid pasteurized eggs from vitamin E fed chickens; b) brand 1 of whole eggs from vitamin E fed chickens; c) brand 2 of whole eggs from vitamin E fed chickens; and d) whole eggs from chickens fed ordinary feed (control eggs). To measure the whole eggs two eggs were broken and the white and yolk homogenized. Twenty five ml of homogenized egg was diluted 1:2 with water and 25 ml of the resulting solution was tested as above. For the liquid eggs 25 ml

was diluted 1:2 with water and 25 ml was measured as above. The results are shown in Table 5

Table 5.

Egg Sample	Corrected IRU/ml
a	2190
b	2675
c	2645
d	932

These results confirm that vitamin E supplementation of chicken feed does result in eggs with significantly higher levels of antioxidant.

Example 6; Antioxidant Level of "Health Supplements"

A variety of herbal supplements are currently touted as being especially rich in antioxidant properties. A number of herbal preparations were obtained and measured by the current method. One half gram of each supplement was suspended in 100 ml of deionized water (final concentration of 5 mg/ml assuming the supplements were fully water soluble). The corrected IRU values are given in Table 6, below.

Table 6.

Herbal Preparation	Corrected IRU/ml
green tea	520
saw palmetto	29
kava kava	-14
Gingko biloba	480
korean ginseng	18

These results show that the two materials widely believed to be especially high in antioxidants (green tea and *Gingko*) do, in fact, test high with the current method. The negative value of kava kava suggests that this material has little antioxidant detectable by the current method. Therefore, "instrument noise" may result in a negative value when subtractive corrections are made. Similarly, the values for saw palmetto and ginseng are so low as to be suspect.

The preferred way of practicing the current method is to measure iodide ion particularly with an ion sensitive electrode. Chemical methods of measuring iodide could also be used but would not be a convenient (or probably as accurate) as the ion electrode. Similarly, the method could be practiced by measuring the decrease in iodine as opposed to the increase in iodide. Again quantitative chemical methods could be employed but this would generally complicate the method and detract from its usefulness. Iodine can also be detected colorimetrically (e.g., spetrophotometrically) by extracting it into organic solvent. In this mode a rough concentration estimate can be made by comparing the resulting purple color to a standard card. While not as accurate as an iodide electrode such an approach does allow the current method to be practiced with minimal equipment.

In addition to the equivalents of the claimed elements, obvious substitutions now or later known to one with ordinary skill in the art are defined to be within the scope of the defined elements. The claims are thus to be understood to include what is specifically illustrated and described above, what is conceptually equivalent, what can be obviously substituted and also what essentially incorporates the essential idea of the invention. Those skilled in the art will appreciate that various adaptations and modifications of the just-described preferred embodiment can be configured without departing from the scope and spirit of the invention. The illustrated embodiment has been set forth only for the purposes of example and that should not be taken as limiting the invention. Therefore, it is to be

understood that, within the scope of the appended claims, the invention may be practiced other than as specifically described herein.

CLAIMS

What Is Claimed Is:

1. A method for determining a level of antioxidant in a liquid sample comprising the steps of:

contacting the sample with an aqueous solution of elemental iodine and an iodophor to form a mixture; and

measuring a change in a concentration of iodide ions in the mixture wherein the change corresponds to the level of antioxidant.

2. The method of Claim 1, wherein the iodophor is polyvinylpyrrolidone.

3. The method of Claim 1, wherein the step of measuring measures an increase in the concentration of iodide ions by means of an ion selective electrode.

4. A method for determining a level of antioxidant in an aqueous liquid sample comprising the steps of:

contacting the sample with an aqueous solution of elemental iodine and polyvinylpyrrolidone to form a mixture; and

measuring an increase in a concentration of iodide ions in the mixture by means of an iodide selective electrode wherein the increase corresponds to the level of antioxidant.

5. A method for determining a level of antioxidant in an sample comprising the steps of:

contacting the sample with a solution of elemental iodine; and

measuring a change in a concentration of iodide ions, wherein the change corresponds to the level of antioxidant.

ABSTRACT OF THE DISCLOSURE

A simple analytical method for determining antioxidant level in food product and body fluids such as urine is based on reduction of elemental iodine. The method adds an aqueous solution of iodine and an iodophor to the sample to be tested. Polyvinylpyrrolidone is a preferred iodophor. Antioxidant materials in the sample reduce the elemental iodine and the reaction is monitored by measuring either a decrease in iodine or an increase in iodide ion. A preferred method of practicing the invention is to measure the change in iodide ion with an ion selective electrode and an appropriate electronic meter. The method rapidly and inexpensively produces antioxidant measurements that are comparable to those produced by my more complex and cumbersome methods.

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COMBINED POWER OF ATTORNEY
DECLARATION AND PETITION

As the below-named inventor, I hereby declare that:

My residence, post office address and citizenship is as stated below next to my name.

I believe that I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD FOR QUANTIFYING ANTIOXIDANT LEVELS IN FOODS AND MEDICAL SPECIMENS, the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed: NONE

PRIOR FOREIGN APPLICATION(S)

Number	Country	Date Filed	Priority claimed Yes	No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below: NONE

Application Serial No. Filing Date

Application Serial No. Filing Date

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application: NONE

Application Serial No.	Filing Date
Application Serial No.	Filing Date

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

David L. Fehrman, Reg. No. 28,600 David B. Abel, Reg. No. 32,394 Vincent J. Belusko, Reg. No. 30,820 Brian M. Berliner, Reg. No. 34,549 David L. Henty, Reg. No. 31,323 Stuart L. Merkadeau, Reg. No. 33,262 Wayne M. Smith, Reg. No. 42,160	Victor De Gyarfas, Reg. No. 40,583 Stefan J. Kirchanski, Reg. No. 36,568 Alma P. Levy, Reg. No. 43,751 Hisako Muramatsu, Reg. No. 34,955 Martin M. Noonen, Reg. No. 44,264 David T. Yang, Reg. No. 44,415

all attorneys of the law firm of Graham & James LLP, 801 So. Figueroa St., 14th Floor, Los Angeles, CA 90017-5554 as my attorneys with full powers of substitution and revocation to prosecute this application and to transact all business in the United States Patent and Trademark Office in connection therewith.

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Wherefore I pray that Letters Patent be granted to me for the invention or discovery described and claimed in the foregoing specification and claims, and I hereby subscribe my name to the foregoing specification and claims, declaration and petition.

Full name of sole inventor: Edward Shanbrom

Inventor's signature: Edward Shanbrom

Date: May 14, 1999

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